Gene Expression Profiling of Xenobiotic Metabolizing Enzymes (XMEs) in the Aging Male Fisher Rat

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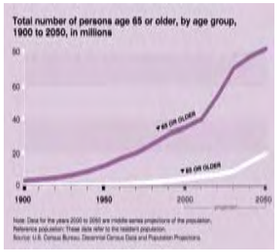
Abstract

Detoxification and elimination of xenobiotics is a major function of the liver and is important in maintaining the metabolic homeostasis of the organism. The degree to which aging affects hepatic metabolism is not known. The expression of XMEs, in part, determines the fate of the xenobiotic and whether exposure will result in toxicity. Gene expression profiles for XMEs in male Fisher rats (6, 11, 18, 24 months of age) were generated using Affymetrix Rat 230 2.0 arrays. Principal component analysis showed a clear age-dependent separation in expression profiles between 6 and 24 month hepatic transcripts. Differentially expressed genes (DEG) were identified using the following algorithm: background correction was performed using MAS5.0 followed by a quantile normalization, perfect match adjustment, median polish, LOESS normalization and Cyber T statistics. 1135 genes were found to be significantly altered in a 24 versus 6 month comparison, and 155 genes were significantly altered in a 18 versus 6 month comparison. No significant gene changes were observed between 11 and 6 month old rats. In the 24 versus 6 month comparison, we found 21 phase I, 23 phase II, and 45 phase III metabolism genes significantly altered. In the 18 versus 6 month comparison, we found 7 phase I, 6 phase II, and 27 phase III metabolism genes significantly altered. qRT-PCR was performed to confirm altered expression. These data confirm an age-dependent change in XME gene expression in male Fisher rats. This information can be used to adapt pharmacokinetic models to reflect age-dependent differences in xenobiotic metabolism.

Introduction

In the United States today, over 12% of the population is over the age of 65, and it is estimated that this percentage will increase to nearly 20% by the year 2030. Although advances in medicine and better nutrition have led to an increase in longevity, the growing population of older Americans remain a subpopulation with potential for greater susceptibility to adverse health effects from exposure to environmental pollutants. This project is being conducted under the auspices of the U.S. EPA's Aging Initiative, which was launched in 2002 to help guide research efforts to protect the health of older adults.



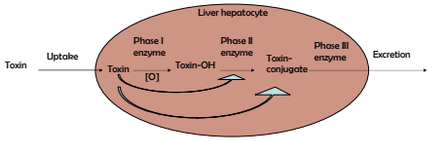
Research goals

- To improve pharmacokinetic models of the aged by incorporating transcript profile information on the differences between young and old populations for xenobiotic metabolism gene expression
- To predict classes of chemicals to which the aged are particularly sensitive
- To provide scientific support for risk assessments that consider susceptible subpopulations

Hypothesis

Pharmacokinetic models will likely be more biologically realistic changes in key xenobiotic metabolizing enzymes (XMEs) under a broad range of conditions or ages could be incorporated

Xenobiotic metabolism



- Phase I (mainly monooxygenases) convert hydrophobic chemicals into hydrophilic chemicals
- Phase II (UGTs, SULTs, GSTs, NATs) further convert these products into amphiphilic anionic conjugates
- Phase III (transporters) export products out of the hepatocyte

Materials and methods

This project was designed to examine the changes in XMEs during the aging process in male Fisher rats (6, 11, 18, 24 months of age). Tissues were selected based on minimal histopathology. In a parallel study, we also looked at changes in male Brown Norway rats (4, 12, 24 months of age). Total RNA was isolated from rat livers according to the TRIzol RNA extraction protocol (Invitrogen) followed by RNeasy Mini Kit (QIAGEN). RNA quality was assessed using a bioanalyzer (Agilent), and RNA quantity was determined using Nanodrop. Total RNA (5 µg) was labeled using the Affymetrix® One-Cycle cDNA Synthesis protocol and hybridized to Affymetrix® Rat 230 2.0 arrays as described by the manufacturer.

Four Fisher rats and three Brown Norway rats per age group were profiled.

Differentially expressed genes (DEG) were identified using the following algorithm:

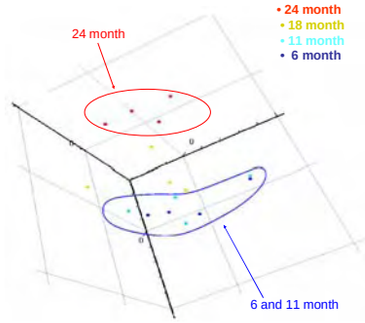
Background correction (MAS5.0)
↓
Quantile normalization (RMA)
↓
Perfect match adjustment (MAS5.0)
↓
Median polish (RMA)
↓
LOESS normalization
↓
Cyber T statistics

Fold change of 1.5 and p-value ≤ 0.05 was used as cutoff.

qRT-PCR using Taqman® probes was performed to confirm altered expression. 9 genes were used to confirm microarray results. Fluorescence was measured using the ABI PRISM® 7700 Sequence Detection System.

Results

Principal Component Analysis (PCA)



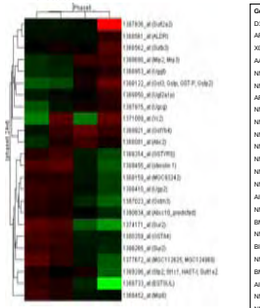
- There is a clear separation between 24 month old animals and younger animals.

Differentially expressed genes- 24 versus 6 month:

Phase I

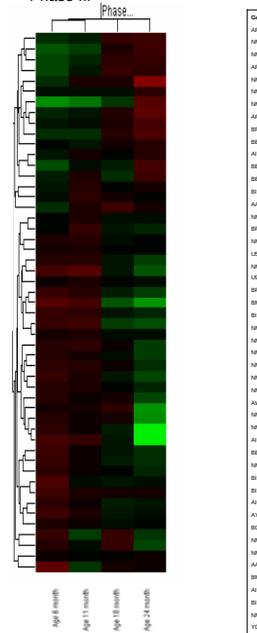
Genbank	Gene	Fold change
NM_031572	Cyp2c40	19.4
M23995	Aldh1a4	2.67
A454611	Fmo5	2.35
AA997683	Aldh1b1_predicted	2.28
NM_012580	Hmox1	1.99
NM_013197	Alas2	1.81
NM_012692	Cyp2a1	1.79
NM_133586	Ces2	1.52
NM_031241	Cyp8b1	-1.54
AA859049	Es2	-1.56
K02422	Cyp1a2	-1.6
BF392959	Cyp2u1_predicted	-1.89
L46791	Ces3	-1.89
AA893326	Cyp4a14	-2.08
U48219	Cyp2d22	-2.11
NM_012792	Fmo1	-2.14
U48220	Cyp2d22	-2.32
BE185541	Cyp26b1	-2.61
BF397093	Cyp26b1	-2.67
J02861	Cyp2c13	-3.78
NM_012693	Cyp2a2	-11.5
U09742	Cyp3a11	-18.46
NM_019184	Cyp2c	-18.5

Phase II



Genbank	Gene	Fold change
D14989	Sult2a2_predicted	14.48
AF072816	Abcc3	3.86
X02904	Gstp1 ; Gstp2	2.73
AA945082	Yc2	2.45
NM_031641	Sult4a1	2.44
NM_033352	Abcd2	2.35
AF047707	Ugcg	2.06
NM_133596	Ugcgl1	1.92
NM_020540	Gstm4	1.65
NM_022228	Ugt2a1	1.62
NM_024396	Abca2	1.57
NM_053293	Gstt1	-1.55
NM_080582	Abcb6	-1.55
NM_031013	Abcc6	-1.55
AI170679	Ugp2_predicted	-1.68
NM_031732	Sult1a2	-1.74
BM385856	Gstm2	-1.8
NM_031154	Gstm3	-1.81
BI300997	Sult1c2	-2.18
NM_013040	Abcc9	-2.77
BM390369	Abcc10_predicted	-3.03
AI170507	Abcc9	-3.43
NM_053754	Abcg5	-5.03
NM_012883	Ste	-22.75

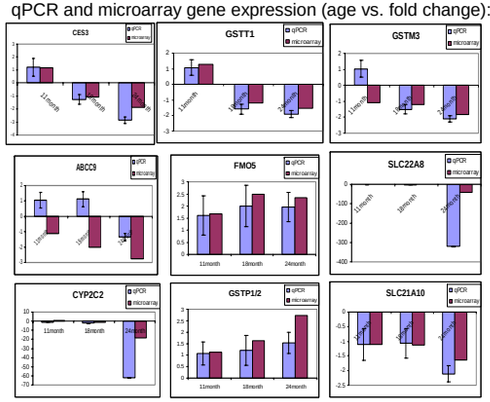
Phase III



Genbank	Gene	Fold change
AF072816	Abcc3	3.86
NM_053859	Slc17a7	3.21
NM_053380	Slc34a2	3.09
AF210250	Slc4a4	2.85
NM_134179	U57.4	2.58
NM_033352	Abcd2	2.35
NM_017353	Slc7a5	2.28
AF276870	Slc38a5	2.05
BF392130	Slc4a4	2.04
BE108272	Slc7a6_predicted	1.88
AI229864	Slc40a1	1.7
BE113959	Slco5a1_predicted	1.68
BE113640	Slc4a1	1.67
BI294340	Slc39a14_predicted	1.64
AA859652	Slc16a6	1.63
NM_133315	Slc40a1	1.6
BF416861	Slc4a4	1.6
NM_024396	Abca2	1.57
U51153	Slc13a2	-1.5
NM_021594	Slc9a3r1	-1.5
U65011	Slco1a4	-1.5
BF283298	Slc19a2_predicted	-1.51
BM392280	Slc29a3	-1.52
BI290895	Slc43a1_predicted	-1.52
NM_133614	Slc25a21	-1.53
NM_031741	Slc2a5	-1.54
NM_131906	Slco1a4	-1.55
NM_080582	Abcb6	-1.55
NM_031013	Abcc6	-1.55
NM_031684	Slc29a1	-1.6
AW144020	Mrp81_predicted	-1.62
NM_031650	Slc21a10	-1.64
NM_031589	Slc37a4	-1.64
AI177358	Slc25a25	-1.72
BE113348	Mrp14_predicted	-1.83
NM_053494	Slc2a8	-1.88
BI294864	Slc10a4_predicted	-1.97
BI276649	Slco5a1_predicted	-2.03
AI045884	Mrp32_predicted	-2.13
AY009158	Slc24a3	-2.43
BG381311	Slc13a5	-2.51
NM_017222	Slc10a2	-2.56
NM_013040	Abcc9	-2.77
AA799760	Slc2a4	-3
BM390369	Abcc10_predicted	-3.03
AI170507	Abcc9	-3.43
BI286134	Slc27a4_predicted	-3.82
NM_053754	Abcg5	-5.03
Y09945	Ugt1r	-5.2
NM_134380	U57.5r	-14.87
NM_031332	Slc22a8	-40.25

Real time PCR results

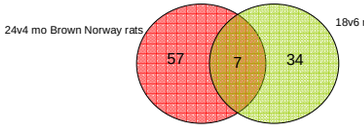
qPCR and microarray gene expression (age vs. fold change):



Comparison to Brown Norway rats

To account for differences in aging survival rates, we compared 24 month old Brown Norway rats to 18 month old Fisher rats.

XMEs:



genbank	Gene	Fisher (18v6mo) fold change	Brown Norway (24v4mo) fold change
K02422	Cyp1a2	-1.26	-1.59
NM_012693	Cyp2a2	-1.38	-1.54
U48220	Cyp2d22	-1.42	-1.94
AA997683	Aldh1b1_predicted	-2.18	-3.65
AB000489	Slc20a1	1.53	1.67
NM_133614	Slc25a21	-1.59	-8.94
BG666999	Slc25a4	1.64	1.64
NM_133315	Slc40a1	1.57	2.10
BG378480	Slc6a6	-1.88	-1.96
M95413	Slc6a9	2.39	2.88
AF200684	Slc7a7	1.47	1.96
NM_131906	Slco1a4	-1.34	-1.55

Conclusions

These data suggest an age-dependent change in XME gene expression in male Fisher rats. This includes genes regulated by AhR (CYP1A), CAR and PXR (CYP2 and CYP3). Gene expression data shows differences in metabolism and elimination between young and old animals. By comparing this Fisher rat dataset to a Brown Norway rat dataset, we have identified common changes in XMEs during aging across strains. Our results can be used to make predictions on susceptibility to environmental chemicals in the aging population based on known interactions between chemicals and the altered XMEs. The next step is to integrate XME gene expression behavior in PBPK models of different life stages that help to predict toxicity in different subpopulations.

Acknowledgments

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